

WHAT IS CLAIMED IS:

1 1. A method of analyzing a target polynucleotide comprising:
2 (a) providing a primed target polynucleotide attached to a
3 microfabricated synthesis channel;
4 (b) flowing a first nucleotide through the synthesis channel under
5 conditions whereby the first nucleotide attaches to the primer, if a complementary nucleotide
6 is present to serve as template in the target polynucleotide;
7 (c) determining presence or absence of a signal, the presence of a
8 signal indicating that the first nucleotide was incorporated into the primer, and hence the
9 identity of the complementary base that served as a template in the target polynucleotide;
10 (d) removing or reducing the signal, if present; and
11 (e) repeating steps (b)-(d) with a further nucleotide, the same or
12 different from the first nucleotide, whereby the further nucleotide attaches to the primer or a
13 nucleotide previously incorporated into the primer.

1 2. The method of claim 1, wherein
2 step (a) comprises providing a plurality of different primed target
3 polynucleotides attached to different synthesis channels;
4 step (b) comprises flowing the first nucleotide through each of the
5 synthesis channels; and
6 step (c) comprises determining presence or absence of a signal in each
7 of the channels, the presence of a signal in a synthesis channel indicating the first nucleotide
8 was incorporated into the primer in the synthesis channel, and hence the identity of the
9 complementary base that served as a template in the target polynucleotide in the synthesis
10 channel.

1 3. The method of claim 2, wherein step (a) comprising providing a
2 plurality of different primed target polynucleotides attached to each synthesis channel.

1 4. The method of claim 1, wherein said first nucleotide and said further
2 nucleotide are labeled.

1 5. The method of claim 1, further comprising flushing the synthesis
2 channel to remove unincorporated first or further labeled nucleotide.

1 6. The method of claim 4, wherein steps (b)-(d) are performed at least
2 four times with four different types of labeled nucleotides.

1 7. The method of claim 4, wherein steps (b)-(d) are performed until the
2 identity of each base in the target polynucleotide has been identified.

1 8. The method of claim 4, wherein said synthesis channel is formed by
2 bonding a microfluidic chip to a flat substrate.

1 9. The method of claim 8, wherein said target polynucleotide is
2 immobilized to the interior surface of said substrate in said synthesis channel.

1 10. The method of claim 9, wherein said interior surface is coated with a
2 polyelectrolyte multilayer (PEM).

1 Sub A3 11. The method of claim 8, wherein said microfluidic chip is fabricated
2 with an elastomeric materia.

1 12. The method of claim 11, wherein said an elastomeric material is RTV
2 silicone.

1 13. The method of claim 4, wherein at least one of the labeled nucleotide
2 comprises a mixture of labeled and unlabeled forms of the nucleotide.

1 14. The method of claim 4, wherein cross section of said synthesis channel
2 has a linear dimension of less than $100\text{ }\mu\text{m} \times 100\text{ }\mu\text{m}$, less than $10\text{ }\mu\text{m} \times 100\text{ }\mu\text{m}$, less than 1
3 $\mu\text{m} \times 10\text{ }\mu\text{m}$, or less than $0.1\text{ }\mu\text{m} \times 10\text{ }\mu\text{m}$.

1 15. The method of claim 4, wherein said label is a fluorescent label.

1 16. The method of claim 15, wherein said removing or reducing is by
2 photobleaching.

1 17. The method of claim 4, wherein said label is a radiolabel.

1 18. The method of claim 17, wherein said removing or reducing is by
2 chemical or enzymatic release of the label.

1 19. The method of claim 4, wherein said label is a mass-spectrometric
2 label.

1 20. The method of claim 19, wherein said removing or reducing is by
2 chemical or enzymatic release of the label.

1 21. The method of claim 1, wherein said signal is a non-optical signal.

1 22. The method of claim 21, wherein said non-optical signal is
2 pyrophosphate release.

1 23. The method of claim 22, wherein said pyrophosphate release is
2 detected with mass spectrometry.

1 24. The method of claim 22, wherein said pyrophosphate release is
2 detected with an enzymatic reaction.

1 25. The method of claim 24, wherein said enzymatic reaction is a redox
2 enzymatic reaction.

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1 Sub A4 > 26. A method of analyzing a target polynucleotide comprising:
2 (a) pretreating the surface of a substrate to create surface chemistry
3 that facilitates polynucleotide attachment and sequence analysis;
4 (b) providing a primed target polynucleotide attached to a surface of a
5 substrate;
6 (c) providing a labeled first nucleotides to the attached target
7 polynucleotide under conditions whereby the labeled first nucleotide attaches to the primer, if
8 a complementary nucleotide is present to serve as template in the target polynucleotide;
9 (d) determining presence or absence of a signal, the presence of a
10 signal indicating that the labeled first nucleotide was incorporated into the primer, and hence
11 the identity of the complementary base that served as a template in the target polynucleotide;
12 and
13 (e) repeating steps (c)-(d) with a labeled further nucleotide, the same
14 or different from the first labeled nucleotide, whereby the labeled further nucleotide attaches
15 to the primer or a nucleotide previously incorporated into the primer.

1 27. The method of claim 26, wherein said substrate is glass and said
2 surface is coated with a polyelectrolyte multilayer (PEM).

1 28. The method of claim 27, wherein said PEM is terminated with a
2 polyanion.

1 29. The method of claim 28, wherein said polyanion bears pendant
2 carboxylic acid groups.

1 30. The method of claim 26, wherein said target polynucleotide is
2 biotinylated, and said surface is coated with streptavidin.

1 31. The method of claim 30, wherein said surface is coated with biotin
2 prior to coating with streptavidin.

1 32. The method of claim 31, wherein said surface is coated with a
2 polyelectrolyte multilayer (PEM) terminated with carboxylic acid groups prior to attachment
3 of biotin.

1 33. The method of claim 32, wherein said surface is pretreated with RCA
2 solution prior to coating with said PEM.

1 34. A method of analyzing a target polynucleotide comprising:
2 (a) providing a primed target polynucleotide;
3 (b) providing a first nucleotide under conditions whereby the first
4 nucleotide attaches to the primer, if a complementary nucleotide is present to serve as
5 template in the target polynucleotide; wherein a fraction of said first nucleotide is labeled.
6 (c) determining presence or absence of a signal from the primer, the
7 presence of a signal indicating the first nucleotide was incorporated into the primer, and
8 hence the identity of the complementary base that served as a template in the target
9 polynucleotide; and
10 (d) repeating steps (b)-(c) with a further nucleotide, the same or
11 different from the first nucleotide, whereby the further nucleotide attaches to the primer or a
12 nucleotide previously incorporated into the primer; wherein a fraction of said further
13 nucleotide is labeled.

1 35. The method of claim 34, wherein said label is a fluorescent label.

1 36. The method of claim 35, wherein said removing or reducing is by
2 photobleaching.

1 37. The method of claim 36, wherein said fraction of the first nucleotide
2 and said fraction of the further nucleotide are less than 10%.

1 38. The method of claim 37, wherein said fraction of the first nucleotide
2 and said fraction of the further nucleotide are less than 1%.

1 39. The method of claim 38, wherein said fraction of the first nucleotide
2 and said fraction of the further nucleotide are less than 0.1%.

1 40. The method of claim 39, wherein said fraction of the first nucleotide
2 and said fraction of the further nucleotide are less than 0.01%.

1 41. An apparatus for analyzing the sequence of a polynucleotide,
2 comprising:

- 3 (a) a flow cell comprising at least one microfabricated synthesis channel; and
4 (b) an inlet port and an outlet port, said inlet port and outlet port being in fluid
5 communication with said flow cell for flowing fluids into and through said flow cell.

1 42. The apparatus of claim 41, further comprising a detector to detect a
2 signal from said surface.

1 43. The apparatus of claim 42, further comprising a light source to
2 illuminate the surface of said synthesis channel.

1 44. The apparatus of claim 42, wherein said signal is a fluorescent signal.

1 45. The apparatus of claim 42, wherein said signal is an electrochemical
2 signal.

1 46. The apparatus of claim 41, wherein said synthesis channel is formed by
2 bonding a microfluidic chip to a substrate.

1 47. The apparatus of claim 46, wherein said microfluidic chip further
2 comprises microfabricated valves and microfabricated pumps in an integrated system with
3 said microfabricated synthesis channel.

1 48. The apparatus of claim 47, further comprising a plurality of reservoirs
2 for storing reaction reagents, wherein said microfabricated valve and said microfabricated
3 pump are connected to said reservoirs.

1 49. The apparatus of claim 41, , wherein cross section of said synthesis
2 channel has a linear dimension of less than $100\mu\text{m} \times 100\mu\text{m}$, less than $10\mu\text{m} \times 100\mu\text{m}$, less
3 than $1\mu\text{m} \times 10\mu\text{m}$, or less than $0.1\mu\text{m} \times 1\mu\text{m}$.

1 50. The apparatus of claim 42, wherein said detector is a photon counting
2 camera.

1 51. The apparatus of claim 46, wherein said microfluidic chip is fabricated
2 with an elastomeric material.

1 52. The apparatus of claim 51, wherein said elastomeric material is RTV
2 silicone.

1 53. The apparatus of claim 52, wherein said substrate is a glass cover slip.

1 54. The apparatus of claim 41, further comprising an appropriately
2 programmed computer for recording identity of a nucleotide when said nucleotide becomes
3 linked to a synthesis channel.